A057 Applicability of CIM® monolithic chromatography in nickel speciation analysis

Convective Interaction Media (CIM®) monolithic chromatographic supports have been successfully introduced in biotechnological industry for purification of large biopharmaceuticals, but can be equally efficient in other application areas. Element speciation analysis requires an efficient and rapid separation of element species and their highly sensitive detection. In order to preserve the original species present in the sample and prevent any species conversion during analysis, fast separation is usually required. Due to fast and high resolution separation, the CIM monolithic chromatographic columns has been gradually introduced for separation of metal-biomolecule complexes.[1-3]

Nickel (Ni) is considered to be a non-essential element for humans, while the food we eat represents an important source of exposure to Ni. Its levels in food and drinks are normally low, but sensitive individuals may develop allergic reactions to Ni as a result of dietary consumption. Cocoa contains relatively high Ni concentrations. However, there is a lack of information about Ni speciation in cocoa.

The application describes separation of Ni species by assembling four weak CIM DEAE anion-exchange disks into a monolithic column. The concentrations of the Ni species eluted from the column were quantified by post-column isotope dilution inductively coupled plasma mass spectrometry (ID)-ICP-MS. The Ni binding ligands eluted under the chromatographic peaks were identified off-line by tandem electro spray mass spectrometry (ESI-MS-MS), scanning for negative ions.

The mild chromatographic conditions of the CIM DEAE disks preserved chemical species and enabled separation of negatively charged Ni complexes.[4] NH_4NO_3 was chosen as eluent since it enabled separation of Ni species and is compatible with ICP-MS and mass spectrometry detectors.

METHOD

Monolithic column:	Four monolithic CIM DEAE 0.34 mL disks in series
Eluent:	0.6 mol L^{-1} NH_4NO_3
Sample load:	0.5 mL of cocoa infusion
Column regeneration:	4 mol L^{-1} NH_4NO_3 0.2 mol L^{-1} HEPES (pH 5)
Column equilibration:	Water
Column cleaning:	1 M NaOH
Detection of Ni separated species:	On-line by post column isotope dilutions ICP-MS
Identification of Ni binding ligands:	Off-line by ESI-MS-MS, scanning for negative ions

The chromatographic run consisted of the following steps:

Time (min)	Flow rate (mL min^{-1})	Eluent A (%)	Eluent B (%)	Eluent C (%)	Step	Eluent forwarded to
0.0	1.0	100	0	0	Separation	ICP-MS
10.0	1.0	85	15	0		
10.1	10.0	0	100	0	Regeneration	Waste
11.0	10.0	0	100	0		
11.1	10.0	0	0	100	Regeneration	Waste
13.0	10.0	0	0	100		
13.1	10.0	100	0	0	Equilibration	Waste
24.0	10.0	100	0	0		
24.1	1.0	100	0	0	Equilibration	ICP-MS
25.0	1.0	100	0	0		

A: water
B: 4 mol L^{-1} HN_4NO_3
C: (0.2 mol L^{-1} HEPES, pH 5)

Note: To obtain reproducible chromatographic separations it is of paramount importance that after regeneration with 4 mol L^{-1} HN_4NO_3, the regeneration with strong buffer solution (0.2 mol L^{-1} HEPES, pH 5) follows, to ensure that the column support has the same pH as the eluent used for separation.

CLEANING PROCEDURE

After approximately 30 successive separations, the column is cleaned by pumping 20 mL of 1 mol L^{-1} NaOH through the column at a flow rate of 1 mL min^{-1}. The NaOH is rinsed from the column at a flow rate of 10 mL min^{-1} by the consecutive application of 50 mL of water, 50 mL of 0.2 mol L^{-1} HEPES (pH 5), 20 mL of 2 mol L^{-1} NaCl, 50 mL of 0.2 mol L^{-1} HEPES (pH 5) and finally by 50 mL of water.

RESULTS

The separation of the Ni species in the raw cocoa nibs infusion was performed by assembling up to four disks into a single housing, so increasing the capacity of the monolithic column. The separated Ni species were detected by ICP-MS. The chromatograms are presented in Figure 1. As evident, Ni is eluted in three chromatographic peaks. It can be further seen that an increasing number of disks (higher column capacity) improved the chromatographic selectivity. Therefore, in all further experiments, four disks were assembled into a CIM monolithic column.

The peak that was eluted with the solvent front (50 to 150 s) was assumed to be the Ni^{2+} species. As citric acid is present in the cocoa pulp and is the predominant acid in fresh cocoa seeds, and was by ESI-MS also identified as the prevailing organic acid in raw cocoa infusion, it was most likely that one of the Ni species eluted during the chromatographic separation corresponds to Ni-citrate. Since gluconic acid was also identified by ESI-MS as an important organic acid in raw cocoa infusion, and because during fermentation by micro-organisms gluconic acid is produced from glucose, Ni-gluconate was expected to be one of the possible Ni species. In order to verify the behaviour of Ni^{2+}, Ni-citrate and Ni-gluconate on CIM DEAE column with ICP-MS detection, synthetic standard solutions of these species (Ni concentration 30 ng mL^{-1}) were prepared and the chromatograms overlaid with the chromatogram of the cocoa infusion (Figure 2).

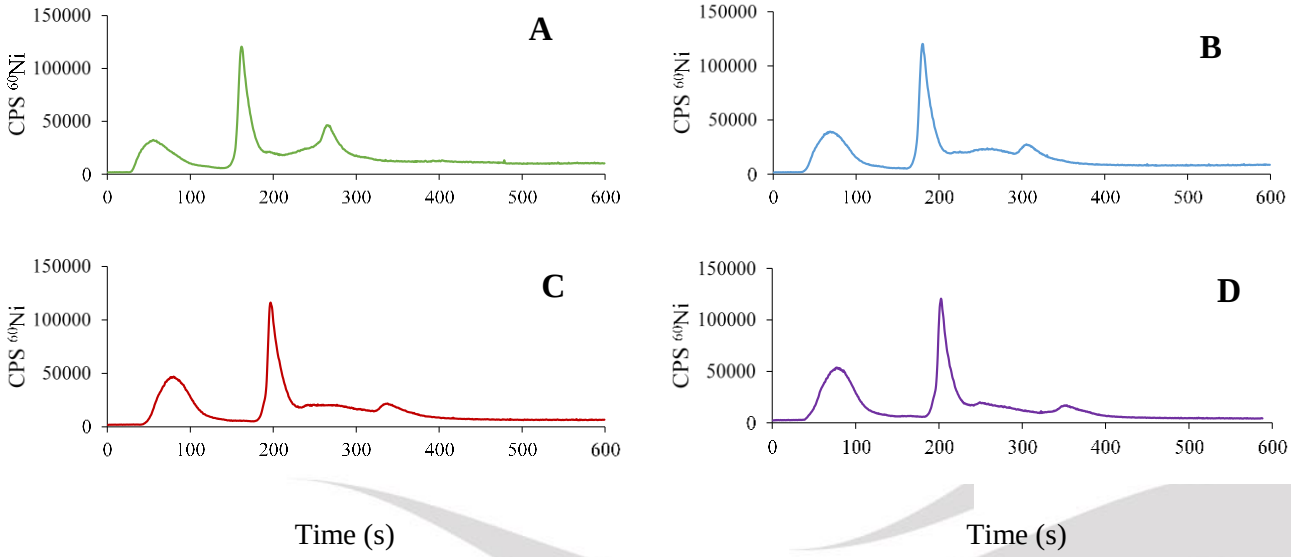

Figure 1. Chromatograms of different Ni-species in cocoa infusions (Raw cocoa nibs of Cacao XP) by CIM DEAE–ICP-MS procedure assembling (A) one disk, (B) two disks, (C) three disks and (D) four disks into a monolithic column.[4]

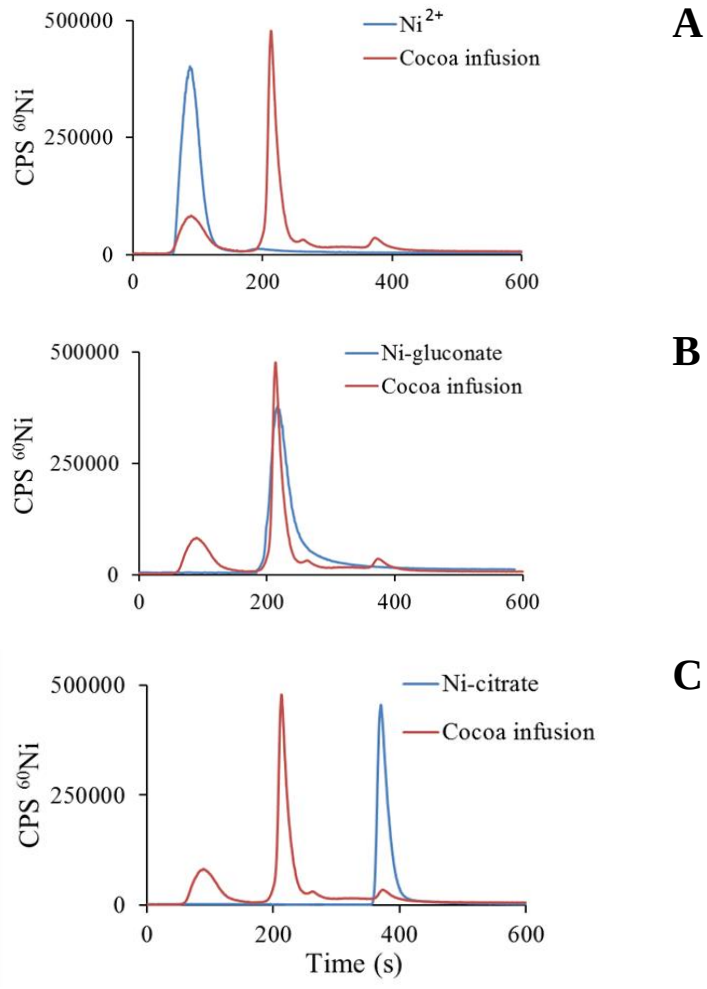

Figure 2. Overlays of chromatograms of standard solutions of Ni species (30 ng Ni mL^{-1}) (A) Ni^{2+}, (B) Ni-gluconate and (C) Ni-citrate, with cocoa infusion (Ekolife natura cocoa powder) by CIM DEAE–ICP-MS procedure.[4]

In order to identify the Ni species, the chromatographic peaks of the synthetic standard solutions and cocoa infusions that contained Ni were collected and subjected to mass spectrometry analysis. MS spectra and daughter MS-MS spectra of the parent ions as well as data from HR-MS analysis confirmed that Ni species present in cocoa infusions correspond to Ni^{2+}, Ni-gluconate and Ni-citrate, of which Ni-gluconate prevailed.

CONCLUSIONS

CIM DEAE monolithic chromatography coupled to ICP-MS was found to be an efficient and reliable analytical tool for separation and quantification of Ni species in cocoa infusions.

The mild chromatographic conditions of the CIM DEAE disks preserved chemical species and enabled separation of negatively charged Ni complexes from Ni^{2+}.

Separated Ni species were identified by ESI-MS-MS and HR-MS. The prevailing Ni species in cocoa infusions was found to be Ni-gluconate, while Ni^{2+} and Ni-citrate were also identified.

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